anols against malaria may also involve interference with biochemical radical processes. The unusual photosensitivity of these compounds could also have an explanation in terms of the ability of the ring system to stabilize a free radical. While attenuation of the radical-stabilizing ability of the ring may decrease photosensitivity, this might also reduce toxicity to the parasites. Quinine itself contains the benzyl alcohol moiety.

If the above picture of the structure–activity relationship in chloramphenicols is correct, some qualitative evidence should be apparent in the activity of some of the other derivatives which have been tested. An interesting example in this connection is that of the 4-phenyl derivative which has been shown to be very active.[32] High activity for this compound is not predicted by electron withdrawal by the phenyl group in terms of σ. However, the phenyl group has been shown to have considerable radical-stabilizing activity.[33]

(32) M. C. Rebstock, C. D. Stratton, and L. L. Bambos, *J. Amer. Chem. Soc.*, **77**, 24 (1955); see also ref 2, p 123.
(33) G. H. Williams, *Chem. Ind.* (London), 1286 (1961).

A point of great importance is that chloramphenicols inhibit strongly the division of HeLa cells. Since the analysis of this report implicates radicals in the process, it would seem worthwhile to study more lipophilic chloramphenicols as antitumor agents, particularly for rapidly growing tumors.

The results with the chloramphenicols underline the usefulness of the extrathermodynamic approach to medicinal structure–activity problems. They also show the difficulty of untangling substituent effects by means of regression analysis. One must be careful to consider a variety of different electronic and steric effects. In fact, it is quite difficult to know when one has exhausted the possibilities. The chloramphenicol series again points out the utmost importance of having highly precise measurements of biological activity if one is to uncover subtle structural features of importance.

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Structure–Activity Relationships among Substrates for a Rabbit Kidney Reductase. Quantum Chemical Calculation of Substituent Parameters

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All-valence electron calculations have been made for a series of substituted acetophenones which are substrates for a rabbit kidney reductase. Substituent constants based on several different molecular parameters have been derived and compared with the relative substrate efficiency of the compounds in the series. Significant correlations using simple linear models for regression analysis have been obtained for properties as inclusive as the relative total energy differences between the ground-state and incipient-transition-state models of the compounds examined.

The isolation and partial purification of a TPNH-dependent carbonyl reductase from rabbit kidney tissue have been described earlier.[1] It was of interest to us to examine the substituent effects among substituted phenacyl derivatives employed as substrates for this enzyme. Some initial results of correlation attempts using the π–ρ–σ approach have been reported.[2] A relatively impure enzyme preparation was used to obtain V_{max} kinetic data for a series of *meta*- and *para*-substituted acetophenones in that study. A significant correlation was demonstrated for the relationship

$$\log V_{max} = k\pi + \rho\sigma + k'$$

where the π term has the meaning assigned by Hansch and Fujita[3] and the $\rho\sigma$ term is that of Hammett.[4,5]

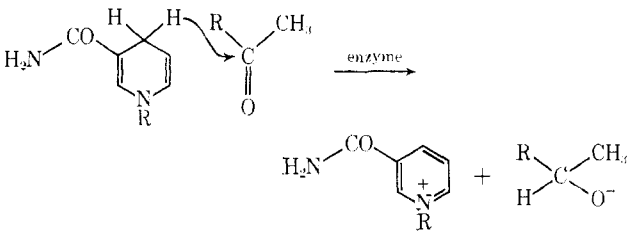
In the present study, more definitive consideration is given to the reaction mechanism and an effort is made to calculate directly the substituent parameter most logically involved in substituent effects on substrate efficiency.

The reductions of the aromatic carbonyl compounds offer a unique opportunity to examine on a quantum chemical basis the relative effect of substituent variation on (a) molecular properties such as orbital charges at a reactive center in the molecule, (b) frontier orbital energies, and (c) electron density in space near a point of reactant attack. Relative energy relationships which can be approximated by molecular orbital methods may also have application in ranking reactivities.

The experimental results reported here represent the reaction situation wherein the elements of a hydride ion are transferred from TPNH to the substituted acetophenone at its carbonyl carbon atom position (C_c) (Scheme I). Such mechanisms for the reduction of carbonyl compounds have been studied both for DPNH- and TPNH-requiring enzymes.[1,6,7] Thus, it would appear intuitively that intramolecular electronic charge distributions which provide a lowered electron density near the carbonyl carbon atom would directly relate to improved substrate efficiency. The observed positive

(1) H. W. Culp and R. E. McMahon, *J. Biol. Chem.*, **243**, 848 (1968).
(2) R. E. McMahon, H. W. Culp, and M. M. Marsh, Abstracts of Papers, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965.
(3) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
(4) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p 186.
(5) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).
(6) C. H. Blomquist, *Acta Chem. Scand.*, **20**, 1747 (1966).
(7) J. M. H. Graves, A. Clark, and H. J. Ringold, *Biochemistry*, **4**, 2655 (1965).

SCHEME I
ENZYMATIC REDOX REACTION INVOLVING DPNH OR TPNH AND A KETONE



coefficient (ρ) for σ in the previously described correlation equation is consistent with this view.[2]

Some of the calculations reported here were made on the ground-state configurations of acetophenone and its derivatives. In this context, it was anticipated that in the enzyme–coenzyme–metal ion-substrate complex the *relative* electronic structure for this series of compounds at the spatial location indicated would not be significantly altered from the ground state by the environment, *or, if altered, would be consistently so throughout the series.* Such an assumption becomes most tenuous when extended to molecular modifications other than substitution on the phenyl nucleus, but within the substituted phenyl series seems reasonable.

Other calculations involved the relative behavior of the potential field of the molecules in the series at a point of approach to the reactive center by a hydride ion.

Rate Constant Determinations.—The enzyme preparations used in these studies were obtained according to the procedures described by Culp and McMahon.[1] The crude enzyme fractions were those which were recovered from ammonium sulfate treatment while the purified ones were carried on through the DEAE-cellulose and alumina gel chromatography steps.

The results from two enzyme preparations are reported here. Their relative activities are expressed in Table I in terms of the logarithms of the rate constants (k_0) or ($k_0 \times 100$) for the various substrates included in this study. The rate constants (k_0) are the pseudo-zero-order constants expressing the rate of loss of TPNH with time extrapolated to zero time. The experimental details of the velocity measurement system have been described earlier.[1]

TABLE I

	Crude enzyme --Log ($k_0 \times 100$)--		Purified enzyme ----Log k_0----	
Substrate	Calcd[a]	Obsd	Calcd[a]	Obsd
Acetophenone	1.111	1.343	1.070	1.068
4-Acetyl-	1.807	2.537[b]	1.775	2.597[b]
4-Nitro-	2.891	2.883	2.872	2.944
4-Acetamido-	1.053	0.634	1.011	0.591
3-Methoxy-	1.372	1.690	1.334	1.613
3-Nitro-	2.495	2.114	2.473	1.965
4-Methyl-	.966	1.398	.923	1.628
4-Methoxy-	1.140	0.204	1.099	0.204
4-Trifluoromethyl-	2.491	2.505	2.468	2.500
3-Methyl-	1.094	1.114	1.052	0.973

[a] Calculations for crude enzyme are from eq 6 and calculations for purified enzyme are from eq 9, Table V. [b] Log ($k_0/2$) since this compound is bifunctional.

The substituted acetophenones were used without purification when gas chromatography of the samples indicated one component or only minor contamination. In general, the compounds were examined on a Barber-Coleman Model 10 gas chromatograph with Argon ionization detector, using a 5% XE-60 silicone stationary phase at 100° or less.

Molecular Geometry. For the purpose of defining a substituent effect on the electron density near C_e, or on any other calculated molecular property, acetophenone was considered to be the parent compound in the series. In a Cartesian coordinate system, the molecule was oriented so that the planar conjugated system lay in the xy plane (Figure 1).

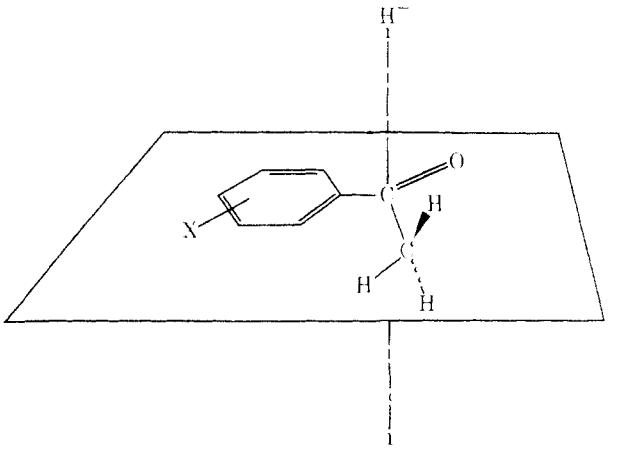


Figure 1.—Assumed incipient transition-state geometry for the reaction of hydride ion with substituted acetophenones.

The electron density in a space element 0.3 Å in the z direction from the C_e nucleus was then calculated for the parent compound and for various *meta*- and *para*-substituted acetophenones used as substrates. The basis for the first set of calculations was extended Hückel theory (EHT) as delineated by Hoffmann.[8] The wave functions computed by the above procedure were then used as input for a computerized calculation of electron density by evaluation at a selected point or points in space.[9]

(8) R. Hoffmann, *J. Chem. Phys.*, **39**, 1397 (1963).

(9) The electron density evaluation at a point P was made in terms of

$$D(\mathrm{P}) = \sum_{i} |\psi_j(\mathrm{P})|^2$$

where

$$\psi_j = \sum_{i}^{\mathrm{A}} \chi_{\mathrm{A}_{ij}} + \sum_{i}^{\mathrm{H}} \chi_{\mathrm{H}_{ij}}$$

χ_{A} is the total orbital density of orbital j on a first-row atom (A) and χ_{H} is the orbital density of orbital j on a hydrogen atom. The first sum is over-all first-row atoms present and the second sum is over-all hydrogens. The evaluation of $\chi_{\mathrm{A}_{ij}}$ for the ith first-row atom is

$$\chi_{\mathrm{A}_{ij}} = \left[a_{ij} \sqrt{\frac{\zeta^5}{96\pi}}\, r e^{-\zeta_i r} + \sqrt{\frac{\zeta^5}{32\pi}} \left(b_{ij} x e^{-\zeta_i r} + c_{ij} y e^{-\zeta_i r} + d_{ij} z e^{-\zeta_i r} \right) \right]$$

and the evaluation of $\chi_{\mathrm{H}_{ij}}$ for the ith hydrogen atom is

$$\chi_{\mathrm{H}_{ij}} = f_{ij} \frac{\zeta^3}{\pi} e^{-\zeta_i r}$$

The probability function, $|\psi_j(\mathrm{P})|^2$ represents the electron density at a defined point (P) in space, due to molecular orbital j. N is the number of atoms in the molecule, ζ_i is the orbital exponent of the ith atom, and f_{ij}, a_{ij}, b_{ij}, c_{ij}, and d_{ij} are the coefficients of the jth molecular orbital on the 1s or the 2s, $2p_x$, $2p_y$, and $2p_z$ orbitals of the ith atom, respectively. The coordinates of the spatial position chosen for evaluation are represented by x, y, and z relative to the atom center around which the electron density is being mapped; $r = (x^2 + y^2 + z^2)^{1/2}$. These coordinates are translated from the initial arbitrarily selected origin.

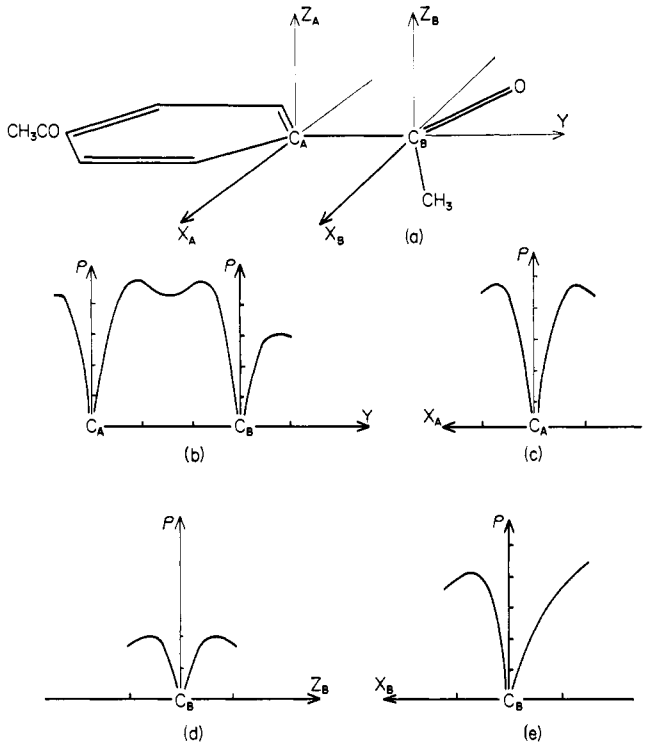


Figure 2.—Electron density from EHT for 4-acetylacetophenone as a representative compound of the series (a). In b, c, d, and e, subdivisions along the electron density axis (ρ) are all 0.05. Subdivisions along the internuclear distance axes are 0.5 Å.

Initially, the electron density around C_c was mapped at 0.1-Å intervals in several directions (Figure 2a–c). The particular location selected for evaluation of the substituent effect appeared to be one of those most sensitive to this effect; in addition, from geometric consideration of the reaction mechanism, it seemed a logical position of influence.

Calculations and Results

I. Extended Hückel Treatment.—The initial molecular orbital concept employed was extended Hückel theory (EHT) as developed by Hoffmann.[8] We have written computer programs implementing this concept for molecules containing up to 98 atomic orbitals (H 1s and the 2s, $2p_x$, $2p_y$, and $2p_z$ orbitals of the other first-row elements). Extended Hückel theory is distinguished from the simpler Hückel-type computational approaches by its inclusion of all valence electrons in the calculations. Atomic parameters used in the calculations are given in Table II.

In order to establish a correlation with substrate efficiency, a series of substituent constants was derived. These (ϵ_c) are analogous to Hammett σ values for the series of aromatic substituent variations among the substituted acetophenones studied. Table III lists the calculated electron densities at the coordinate position (1.54, 0, 0.3) for each of these compounds. The substituent constants (ϵ_c) were obtained by subtracting the value for acetophenone from that for the derivative and, for convenience, multiplying the difference by −1000; this also permits the sign of the substituent constant to have the same significance as the Hammett σ sign.

TABLE II
ATOMIC PARAMETERS[a] FOR MOLECULAR ORBITAL CALCULATIONS

EHT

Element	I_{1s}	I_{2s}	I_{2p}	ζ
H	−13.60			1.00
C		−21.40	−11.40	1.57
O		−35.30	−17.80	2.22
N		−27.50	−14.50	1.915
F		−34.98	−17.42	2.55

CNDO/2

Element	$\left[\frac{(I+A)}{2}\right]_{1s}$	$\left[\frac{(I+A)}{2}\right]_{2s}$	$\left[\frac{(I+A)}{2}\right]_{2p}$	ζ
H	−5.3[b]			
C		−14.051	−5.572	1.625
O		−25.390	−9.111	2.275
N		−19.316	−7.275	1.950
F		−32.272	−11.080	2.60

[a] I = ionization potential, A = electron affinity.[7] [b] The original value in ref 7 was −7.176; this was changed for the reasons indicated under calculation method II.

TABLE III
ELECTRON DENSITIES AND SUBSTITUENT CONSTANTS

	EHT		CNDO/2	
Substrate	Electron density at (1.54, 0, 0.3)	ϵ_c	Electron density at (1.54, 0, 0.3)	ϵ_c
Acetophenone	0.1034	0.0	0.1999	0.0
4-Acetyl-	0.1018	+1.6	0.2003	−0.4
4-Nitro-	0.1000	+3.4	0.2013	−1.4
4-Acetamido-	0.1064	−3.0	0.1992	+0.7
3-Methoxy-	0.1034	0.0	0.2002	−0.3
3-Nitro-	0.1029	+0.5	0.2007	−0.8
4-Methyl-	0.1058	−2.4	0.1995	+0.4
4-Methoxy-	0.1060	−2.6	0.1994	+0.5
4-Trifluoromethyl-	0.1034	0.0	0.2009	−1.0
3-Methyl-	0.1034	0.0	0.2001	−0.2

II. Complete Neglect of Differential Overlap.—Recently, Pople and Segal[10] have developed a molecular orbital treatment (CNDO/2) which takes into account electron repulsions ignored in extended Hückel theory. We have written computer programs for a revised version of this. The revisions are concerned primarily with the need to increase bond moment values for C–H bonds in order to obtain more appropriate inductive effects.[11] The atomic parameters are listed in Table II also. Substituent constants based on electron density for the second correlation approach were derived for the same series as EHT (Table III).

III. Hydride Ion Interaction Energy.—A third concept employed in the structure–activity correlations was the attempt to account for the immediate environment of the reactive center as seen by an approaching hydride ion (Figure 1). Thus an H atom possessing a filled 1s orbital was located 1.68 Å in the z direction above C=O. The position was chosen as a median

(10) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966).

(11) The ability of CNDO/2 to predict the inductive effect of the methyl group was investigated by calculating the relative basicities of the *o*-, *m*-, and *p*-tolyl anions from their relative total energies. The parameterization suggested by Pople fails here while a similar calculation on the anions from pyridine agrees with experiment. It was decided that a change in the parameter of H to give a charge density distribution for the C and H atoms of the CH_3 more nearly like limited basis set calculations on ethane should give a more appropriate C–H dipole. The parameter −5.3 eV was thus chosen for H. The CNDO/2 results then obtained for *m*- and *p*-tolyl anions and pyridyl anions were in agreement with experiment. Certain changes in parameterization will be discussed in a forthcoming paper on the calculation of carbanions by CNDO/2.

location a proton on TPNH might be expected to assume if a complex of the coenzyme and the substrate is formed in the process of reduction of the ketone. With this geometry, a Coulomb integral matrix was generated using CNDO/2. The appropriate elements of the matrix representing the repulsion integrals between the hydride ion and all the atoms of the molecule were selected. These elements, multiplied by the occupation numbers (calculated by CNDO/2) of their respective atoms and summed, represented an approximation of a trend toward a transition state for the reaction. This treatment was carried out for all compounds in the series. Substituent constants were again established by subtracting the parent acetophenone value from that for each derivative. The results are given in Table IV. The correlation as noted for the purified enzyme in Table V was not particularly satisfactory.

TABLE IV
SUBSTITUENT CONSTANTS BASED ON HYDRIDE ION APPROACH (ALL ATOMIC INTERACTIONS)

Compd	Total interaction energy, au	Substituent constant (θ)
Acetophenone	−0.02286	0
4-Acetyl-	−0.02804	0.51
4-Nitro-	−0.04257	1.96
4-Acetamido-	−0.02036	−0.26
3-Methoxy-	−0.02420	0.13
3-Nitro-	−0.05041	2.75
4-Methyl-	−0.02017	−0.27
4-Methoxy-	−0.02341	0.05
4-Trifluoromethyl-	−0.03838	1.55
3-Methyl-	−0.02124	−0.17

difference for the derivative from that for acetophenone and expressing the result as a substituent constant.

TABLE V
COMPARISON OF CORRELATION EQUATIONS[a]

Molecular parameter(s)	Equation	Std error (est'd)	R^2[c]
	Crude Enzyme		
Hammett σ	Log $(k_0 \times 100) = [2.036\ (\pm 0.346)]\sigma + 1.205\ (\pm 0.147)$[b]	±0.400	0.81
Hammett $\sigma + \pi$[c]	Log $(k_0 \times 100) = [0.376\ (\pm 0.096)]\pi + [2.079\ (\pm 0.355)]\sigma + 1.150\ (\pm 0.148)$	±0.386	0.85
Hammett $\sigma + \pi + \pi^2$	Not improved		
Electron density by EHT near carbonyl C[d]	Log $(k_0 \times 100) = [0.375\ (\pm 0.081)]\epsilon_c + 1.736\ (\pm 0.154)$	±0.481	0.73
Electron density by CNDO/2 near carbonyl C[d]	Log $(k_0 \times 100) = [-1.144\ (\pm 0.203)]\epsilon_c + 1.356\ (\pm 0.140)$	±0.413	0.80
Incipient-transition-state energy differences — (CNDO/2)[d]	Log $(k_0 \times 100) = [0.580\ (\pm 0.135)]\delta_E + 1.111\ (\pm 0.203)$	±0.509	0.70
	Purified Enzyme		
Hammett σ	Log $k_0 = [2.040\ (\pm 0.413)]\sigma + 1.170\ (\pm 0.175)$	±0.478	0.75
Hammett $\sigma + \pi$	Log $k_0 = [0.414\ (\pm 0.359)]\pi + [2.088\ (\pm 0.407)]\sigma + 1.110\ (\pm 0.179)$	±0.468	0.79
Hammett $\sigma + \pi + \pi^2$	Not improved		
Occupation numbers by EHT for carbonyl C[d]	Log $k_0 = [0.053\ (\pm 0.014)]\delta_c + 1.693\ (\pm 0.185)$	±0.582	0.63
Electron density by EHT	Log $k_0 = [0.367\ (\pm 0.096)]\epsilon_c + 1.700\ (\pm 0.183)$	±0.573	0.64
Electron density by CNDO/2 near carbonyl C[d]	Log $k_0 = [-1.134\ (\pm 0.247)]\epsilon_c + 1.325\ (\pm 0.171)$	±0.504	0.72
Total interaction energy — hydride ion (CNDO/2)[d]	Log $k_0 = [0.559\ (\pm 0.224)]\theta + 1.259\ (\pm 0.267)$ (not statistically significant)	±0.721	0.44
Eigenvalue differences (LEMO) by CNDO/2[d]	Log $k_0 = [0.346\ (\pm 0.073)]\Delta_E + 1.041\ (\pm 0.196)$	±0.492	0.74
Incipient-transition-state energy differences — CNDO/2[d]	Log $k_0 = [0.588\ (\pm 0.149)]\delta_E + 1.070\ (\pm 0.223)$	±0.559	0.66

[a] All equations were significant ($P = 0.01$) unless otherwise indicated. Statistical validity established on the basis of F test values for the estimate. [b] Numbers in parentheses are standard errors of the coefficients. [c] The π and π^2 terms are those proposed by Hansch and Fujita.[3] [d] Correlation not improved by addition of π^2 and/or π terms. [e] Square of correlation coefficient.

IV. Approaching Transition-State Energies.—The most elaborate approach to the calculation of relative reactivities which we have undertaken involves the comparison of *energy* differences between the ground state and the *approach* to a transition state of method III of each of the compounds in the series. The treatment involved first calculating the electronic energy in the ground state for a substrate molecule using CNDO/2 (convergent to 0.000001 au in electronic energy); then the same calculation was made on the incipient transition state where the molecule remained essentially planar and a hydride ion was positioned 1.68 Å above the C=O (see Calculations, III). The energy difference between the two states was compared with the analogous difference for the parent compound (acetophenone). The comparison again was most simply made for correlation purposes by subtracting the energy The substituent constants obtained are listed in Table VI. It will be recognized that method III is essentially a first-order perturbation on method IV. Method III is a simpler treatment and, if it had been successful, would have been easier to relate to the physical concept of the mechanism of reduction of the substrate. It will be noted that in Table VI, the calculated total molecular energies are less negative for the ground-state configurations than for the perturbed structures which include the hydride ion. However, since the calculations were carried out on the basis of bare molecules and bare ions arbitrarily positioned, there are no thermodynamically based implications of increased stability for these altered structures.

V. Other Molecular Properties.—EHT, CNDO/2, and Hammett σ values were employed to calculate other molecular properties of the compounds in this series.

TABLE VI

SUBSTITUENT CONSTANTS BASED ON ENERGY DIFFERENCES BETWEEN GROUND AND INCIPIENT TRANSITION STATES (CNDO/2)

Substrate	Total energy[a] Ground state	Total energy[a] Incipient transition state	ΔE $(E_{IT} - E_G)$	δ_E[b]
Acetophenone	−80.68633870	−81.22647364	−0.54013	0
4-Acetyl-	−114.67279422	−115.22496167	−0.55217	+1.20
4-Nitro-	−128.58238081	−129.15322663	−0.57085	+3.07
4-Acetamido-	−127.09126212	−127.63035874	−0.53910	−0.10
3-Methoxy-	−107.68248913	−108.22712972	−0.54464	+0.45
3-Nitro-	−128.58296234	−129.14704283	−0.56408	+2.39
4-Methyl-	−89.24733698	−89.78494181	−0.53760	−0.25
4-Methoxy-	−107.65065079	−108.19129665	−0.54065	+0.05
4-Trifluoromethyl-	−170.39296342	−170.95693612	−0.56397	+2.38
3-Methyl-	−89.24658192	−89.78639800	−0.53982	−0.03

[a] Energy in atomic units. [b] Substituent constant (δ_E) = $[-(E_{IT} - E_G)_{compd} + (E_{IT} - E_G)_{acetophenone}] \times 100$, where E_{IT} = total energy of incipient transition state and E_G = total energy of ground state.

The results are summarized in Table V. It can be seen that orbital charges (occupation numbers) by EHT and frontier orbital energies obtained from CNDO/2 correlate reasonably well with reactivity also. This is not unexpected in view of the interdependence of such calculated properties.[12]

VI. Conformational Effects.—A simple comparison of conformational effects among substituents capable of assuming more than one stable conformation was made. In all the calculations reported above, the conformations of all substituents were chosen to give maximum coplanarity with the benzene nucleus; however, the 3-CH_3O and 4-CH_3O substituents were given another set of coordinates corresponding to a conformation of the –OC (methyl) bond perpendicular to the plane of the benzene ring. In addition, the 4-CH_3CONH group was given a conformation in which the peptide –CONH– plane was tilted at about 37° with respect to the benzene ring, as seen from X-ray crystallography of acetanilide.[13] In general, the changes in substituent constants using the total free-energy difference approach for ground state *vs.* incipient transition state were not very large: 4-CH_3O changed from 0.05 (Table IV) to 0.09; 3-CH_3O changed from 0.45 to 0.49; 4-CH_3CONH changed from −0.10 to 0.07.

Discussion

From earlier work with less pure enzyme preparations,[2] we had interpreted a dependence on π to indicate the existence in the preparation of competitive hydrophobic binding sites for the substrate on the protein impurities present. However, the use of substituent constants based on electron densities calculated by EHT in place of the Hammett σ values has eliminated that dependence for most of the tested preparations.

It has been pointed out by Hansch and colleagues[14] that the substituent contribution to the partition coefficient may, in some cases, be related to the Hammett σ. The relationship is complex, and there are undoubtedly several interaction forces comprising π. Solvation by hydrogen bonding, hydrophobic bonding in a structured solvent, and permanent or induced dipole–dipole attractions may be involved.

The Hammett σ value itself may be considered to consist of several components. Dewar and Grisdale[15] have indicated at least five such processes at work: (a) field, (b) σ-bond inductive, (c) π-bond inductive, (d) mesomeric, and (e) electromeric effects. Thus, direct molecular orbital calculations of various molecular properties offer the opportunity, at least, to express the summation of these contributions at a specific position; experimentally derived substituent constants can only infer the summation of these effects in terms of the rates of the specific reaction used to measure the substituent effect. Of course, the particular quantum chemical approach chosen will have a profound effect on the validity of each of the mechanistic contributions to the reactivity index.

It now appears possible to consider that for this substrate series the π dependence could be merely a linear correction term which reflects the inability of the Hammett σ or other molecular parameter to express completely the total electronic effect of the substituent.

It might be expected that self-consistent field (SCF) methods such as CNDO/2 which involve fewer approximations would yield more representative values for electron density variations as a function of the substituent in this series; however, the correlation resulting from the use of substituent constants obtained in this way, while highly significant, does not fit the proposed reaction mechanism model (Table V). We conclude that if the model is appropriate, then CNDO/2 may improperly estimate one or more of the components of the substituent effect in calculating electron density. For obvious reasons, substituent effects based on electron densities calculated by EHT methods are thus also suspect; the observed good correlation could be a fortuitous combination of over- and underestimation of two or more of the total substituent effect components.

The significant feature of the more sophisticated correlation attempts, such as the incipient-transition-state energy treatment, is that one may apply them in the context of a close approach to a fairly complex reaction model and still retain a significant correlation. The desirability of studying variations on a transition-state approach to the expression of reactivity indices has been suggested.[12] An example of energy comparisons made along the reaction coordinate beyond the isolated ground-state geometry has also been reported recently.[16]

Several computer programs were written for the various computational approaches employed in these stud-

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ies. The EHT programs are available for use on IBM 7094 and IBM 360/50 systems. The modified CNDO programs have been used on IBM 360/50 (convergence criterion 0.0001 au in electronic energy) and CDC 3600 and 6500 (0.000001 au) systems. The electron density plotter functions on IBM 360/30 equipment. The program for calculating total interaction energy (HYTRAN) for the hydride ion was written for the CDC 6500 machine.

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Species Difference in the Competitive Binding of 2-(4′-Hydroxybenzeneazo)benzoic Acid (HBABA) and α-(4-Chlorophenoxy)-α-methylpropionic Acid (CPMPA) to Serum Albumin. A Possible Model System for Studying Allosteric Transitions[1a]

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The comparative competitive binding of 2-(4′-hydroxybenzeneazo)benzoic acid (HBABA) and α-(4-chlorophenoxy)-α-methylpropionic acid (CPMPA) with a number of serum albumin preparations obtained from different species was studied. Data derived from equilibrium dialysis, spectrophotometric, and extrinsic rotatory dispersion (ERD) measurements reveal a unique behavior for rat serum albumin (RSA). With serum albumins obtained from all other species studied CPMPA competitively inhibited the HBABA–protein interaction at all concentration levels. With RSA low concentrations of CPMPA actually enhanced the HBABA–protein interaction. These results suggest that CPMPA is able to cause a small molecular perturbation in the RSA molecule which liberates additional sites on the protein for HBABA binding. Rat albumin, in combination with certain drugs, seems to be a model for studying allosteric transitions.

In blood plasma the albumin is a very important constituent because of its relative abundance and homogeneity and also because of its special osmotic and transport properties.[2] In the past, serum albumin was often regarded as a single homogeneous protein with similar physicochemical properties in all species from which the crystalline protein had been isolated. However, exceptions have often been noted and it is now well documented in the literature that species differences do exist in amino acid content[3] and sequence[4] and in the binding of small molecules and ions.[5]

Witiak and Whitehouse[6] have shown that rat serum albumin (RSA) behaves abnormally when compared with plasma albumin fraction V preparations from other species in its interaction with 2-(4′-hydroxybenzeneazo)benzoic acid (HBABA) in the presence of drugs such as thyroxine, indoleacetates, and chlorophenoxyacetic acids and that the rat may be a singularly nonrepresentative species as far as drug binding to its albumin is concerned. This is well known with respect to thyroxine binding. Through results obtained utilizing equilibrium dialysis, spectrophotometry, and optical rotatory dispersion an explanation for this unique effect with RSA is suggested in this study.

HBABA, in 0.1 *M* sodium phosphate buffer, pH 7.4, exhibits a λ_{max} at 350 mμ (ε 19,125), but when combined with serum albumin in phosphate buffer a new λ_{max} of considerably lower intensity is observed at 475–480 mμ.[5a] The intensity of this peak depends on both the concentration of albumin and the amount of HBABA bound. The position of the λ_{max} 475–480 mμ does not change over a range of animal serum albumins, but the actual absorbance at 475 mμ is species dependent.[5a] Assigning a value of 1.0 to the absorbance with bovine serum albumin (BSA), the relative absorbance for other albumins are guinea pig, 1.35; mouse, 1.10; rat, 0.80; human, 0.75; dog, 0.70; sheep, 0.60; rabbit, 0.40; pig, 0.30; horse, 0.10.[6] When various phenoxyacetic acids and indomethacin are added to a phosphate buffer containing RSA and HBABA an increase in absorbance is observed at 475 mμ while phenylbutazone and nicotinic acid quenched the absorbance at all concentration levels. With albumins obtained from all other species[7] that Witiak and Whitehouse[6] investigated, the phenoxyacetic acids decreased the absorbance at 475 mμ.

Optical rotatory dispersion can be utilized in determining the interaction of small molecules with serum albumin. The ORD spectrum has been thoroughly investigated for bovine and human serum albumin[8] and many other polypeptides and proteins. Rotatory parameters derived from such curves have been attributed

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